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14th Annual Workshop

Talk Abstracts

Solution small angle X-ray scattering of elastic fibre proteins

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The extracellular matrices of virtually all connective tissues contain a variety of microfibrillar elements of which fibrillin microfibrils are a major component. These polymers are essential structural elements of matrix and act as a lattice for elastin deposition during elastic fibre formation. Fibrillin microfibrils are extensible polymers which endow connective tissues with long-range elasticity and have widespread distributions in both elastic and non-elastic tissues. They are critically important in maintaining the integrity of tissues such as blood vessels, lung and skin, both in terms of their key roles in linking cells and matrix macromolecules and in the specific biomechanical properties they impart. Linkage of the fibrillin-1 gene to the heritable connective tissue disorder Marfan syndrome, which is associated with severe cardiovascular, ocular and skeletal defects, further highlights its importance.

The molecular pathway of fibrillin microfibril assembly remains poorly understood. Intermediates have proved difficult to identify due to the large size of fibrillin molecules and their propensity to form disulphide-bonded aggregates. Knowledge of the primary structure of fibrillin has also proved difficult to translate into an understanding of supramolecular organisation or mechanical properties. Therefore, we have used small angle X-ray solution scattering to analyse the structure of nine overlapping recombinant protein fragments covering 90% of the 330kDa human fibrillin-1 molecule. We were able to calculate molecular envelopes and perform computational modelling to obtain important structural details that give information of the shape and topology of contiguous groups of domains of fibrillin-1. These data highlight the flexible nature of the proline-rich region, suggesting a role in the molecular basis of fibrillin-1 elasticity. This study has provided new insights into the conformation of tandem repeats of cbEGF domains in solution, the modular structure of fibrillin-1, and the possible arrangement of fibrillin-1 in microfibril organization.

Fibre Diffraction Analysis of Potyviruses

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Potyviruses are flexible filamentous plant viruses, responsible for half the viral crop damage in the world. In addition, they have great potential as vectors in biotechnology. Because of their flexibility and tendency to form disordered aggregates in solution, no fibre diffraction patterns of potyviruses have been published. This is in sharp contrast to the situation for the much more tractable potexviruses and tobamoviruses.

We have obtained disoriented but nevertheless informative diffraction patterns from partially oriented sols of wheat streak mosaic virus. We have obtained more informative diffraction patterns, with well-defined layer lines to beyond 4 %C5 resolution, from dried fibres of bean common mosaic virus (BCMV). Oriented sols were prepared by slow centrifugation in capillary tubes, followed by exposure to strong (up to 18.8 Tesla) magnetic fields. Dried fibres were also prepared in magnetic fields, at ambient humidity and under controlled humidity. Diffraction data were collected at the BioCAT beamline at APS, Argonne. These data have enabled us to determine the most probable symmetry of WSMV and BCMV, and to obtain preliminary information about the internal structure of BCMV.

Virus structure research supported by NSF grant MCB-0235653 and USDA grant 2003-01178. Fibre diffraction methods research supported by NSF Research Coordination Network grant MCB-0234001. Use of the APS supported by the U.S. Department of Energy under contract W-31-109-ENG-38. BioCAT is a NIH-supported Research Center RR-08630.

Introduction to FibreFix - The new Integrated CCP13 software package

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The new CCP13 program FibreFix (for Windows) has been developed to make processing of non-crystalline diffraction patterns simpler for existing users and less intimidating for new users. FibreFix (which supercedes the ICE package) incorporates the functionality of the CCP13 analysis programs XCONV, XFIX, FTOREC and LSQINT and it also includes the diffraction simulation programs HELIX and MusLABEL. This talk will introduce the FibreFix program, it will demonstrate its application to a number of different kinds of diffraction data, and it will provide a background for those wishing to try out the program themselves during the Workshop.

For further details of the CCP13 philosophy behind this program see:
Squire, J.M., AL-Khayat, H.A., Arnott, A., Crawshaw, J., Denny, R., Diakun, G., Dover, S.D., Forsyth, V.T., He, A., Knupp, C., Mant, G., Rajkumar, G., Rodman, M.J., Shotton, M. & Windle, A.H. (2003) New CCP13 software and the strategy behind further developments: Stripping and modelling of fibre diffraction data. Fibre Diffraction Review 11, 7-19.

For further details of the FibreFix program see:
Rajkumar, G., AL-Khayat, H.A., Eakins, F., He, A., Knupp, C. & Squire, J.M. (2005) FibreFix - A New Integrated CCP13 Software Package. Fibre Diffraction Review 13, 11-18.

Ultra small angle scattering at high brilliance beamlines using compound refractive lenses

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Refractive x-ray lenses have recently been applied for imaging and scanning microscopy with hard x-rays. We report the application of refractive lenses in an optical scheme for ultra-small angle x-ray diffraction, performed at a high brilliance synchrotron radiation source. An experimental proof of principle and a theoretical discussion are presented. In particular, we observe the x-ray diffraction pattern from a two-dimensional photonic crystal with 4.2 μm periodicity, which normally is employed to scatter light in the infra-red [1].

[1] M. Drakopoulos, A. Snigirev, I. Snigireva, J. Schilling, Appl. Phys. Lett. 86, 014102 (2005).

Collagen organisation in normal and diseased human cornea

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The cornea, the clear window at the front of the eye, is the principal refractive component of the human visual system. Correct focussing of light onto the retina is highly dependant on corneal shape, which in turn is governed by a layered network of mechanically reinforcing collagen fibrils. We have used wide-angle x-ray fibre diffraction to map the orientation and mass distribution of fibrils over a pair of normal human corneas. The maps indicate that corneal collagen fibrils are organised in a highly specific, anisotropic manner and that, moreover, left and right eyes display structural mirror symmetry about the central body axis. The results have implications for refractive surgery and corneal transplantation. In addition we present equivalent data from eyes with keratoconus, a condition where abnormal collagen organisation is associated with tissue thinning and a conical, astigmatic cornea.

Evolutionary Algorithms *for *Fibre Diffraction Analysis

Dr David Cairns, Dr Graeme Cameron, Prof Tim Wess, Prof Andrew Miller

University of Stirling

Evolutionary algorithms are increasingly being recognised as an important optimisation tool. They provide a very flexible method for locating optimal solutions to complex problems within a realistic time frame. This talk provides an overview of using one family of evolutionary algorithms - the genetic algorithm, to search for optimal solutions which relate to observed X-ray diffraction data from fibrillar collagen. In particular, two studies are presented demonstrating how the general principle can be applied to different problem areas. The first study examines the process of developing an optimal fit for intensities within overlapping Bragg reflections, the second

study looks at the more complex issue of determining axial rise in the molecular structure of collagen using data observed from X-ray diffraction images. The results of the two studies are presented and some general conclusions drawn concerning the suitability of using evolutionary algorithms for fibre diffraction analysis.

A wholly synthetic muscle

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Applications of scattering and reflectivity will be discussed in the context of the development of generic molecular devices based on responsive polymers.

A Landolt pH-oscillator based on a bromate/sulfite/ferrocyanide, with a room temperature period of 20 min and a range of 3.1pH7.0, has been used to drive periodic oscillations in volume in a pH-responsive, polyelectrolyte hydrogel. The gel is coupled to the reaction and changes volume by a factor of at least 6. A continuously stirred tank reactor was set-up on an optical microscope and the reaction pH and gel size monitored. The cyclic force generation of this system has been measured directly in a modified JKR experiment.

The responsive nature of polyelectrolyte brushes, grown by surface initiated ATRP, have been characterised by AFM, neutron reflectivity and single molecule force measurements.

Triblock copolymers, based on hydrophobic end-blocks and either polyacid or polybase, have been used to produce polymer gels where the deformation of the molecules can be followed directly by SAXS and a correlation between molecular shape change and macroscopic deformation has been established. The power developed by these synthetic muscles has been measured.

Chemical contrast by SAXS and NMR in nano-ceramics

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We report here on small angle x-ray scattering experiments performed under in-situ heating conditions at beamline 6.2 at Daresbury, combined with two-dimensional NMR results obtained at UWA. Sol-gel prepared Al2O3 and ZrO2 nanoparticles were mixed with a powdered silicate (Na2Si3O7) glass, pelletised, and heated up to 1000C while acquiring data at photon energies of 8.3keV and near the absorption edge of zirconium, 18keV. Macroscopic grain surface scattering following Porod's law is subtracted from the raw data. The evolution of the scattering pattern reveals changes of the morphology of the interface during the nucleation and initial growth of the particles from sub-nanometer size to a few nm median radius. The roughness of the scattering interfaces, interpreted by a fractal model, decreases during the annealing process. Larger product particles with smoother interfaces are formed above about 550C.

NMR results show that aluminium atoms migrate from tetrahedral site in the nucleating particles through octahedral interface sites towards tetrahedral sites when dissolved in the glass matrix.

The use of NMR (27Al) and near-edge SAXS (Zr K-edge) provides chemical contrast over a wide length range from the atomic to the particle scale, and both techniques are now available as in-situ experiments at high temperature.

Microbeam diffraction from hair and skin

Naoto Yagi, N. Ohta, H. Iwamoto and K. Inoue

SPring-8/JASRI

BL40XU at SPring-8 is called "High Flux beamline" because it uses an undulator radiation without monochromatization [1]. By focusing with two mirrors, the flux density is about 1x10^17 photons/sec/mm^2, which is suitable for a microbeam experiment using a pinhole. With a 5-micron pinhole, the flux is still about 1x10^12 photons/sec. In order to record small-angle diffraction, it is necessary to use a guard pinhole to avoid scattering from the edges of the collimating pinhole. A microbeam with a 5-10 micron diameter is routinely used. The most commonly measured specimens are hair (especially cuticle [2]) and skin (stratum corneum) in which several cosmetic companies are also interested. The microbeam is used for recording small-angel diffraction from single fibres of polymers, myofibrils, and frozen hydrated biological specimens [3]. It is also used in speckle experiments (photon correlation spectroscopy).

1. K. Inoue et al., "Present Status of high flux beamline (BL40XU) at SPring-8." Nucl. Instrum. Meth. A467-468:674-677 (2001).
2. N. Ohta et al. "Structural Analysis of Cell Membrane Complex of a Hair Fibre by Micro-beam X-ray Diffraction." J. Appl. Cryst. 38:274-279 (2005).

3. H. Iwamoto et al. "X-ray microdiffraction and conventional diffraction from frozenhydrated biological specimens." J. Synchrotron Rad. (in press).

High overall throughput detector systems for scattering experiments

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The trend of synchrotron radiation sources is towards producing higher flux x-ray beams. This in turn provides the opportunity for collecting good quality measurements in shorter time periods. Many real-life processes occur quickly and the study of these is well matched to high intensity beams. In order to fully exploit the opportunities offered by newer beamlines, detector systems must be capable of collecting high intensity spots/cones as well as being able to operate at high frame rates. This talk will focus on a detector suite which is being developed for incorporation in the non-crystalline diffraction beamline, I22, on the Diamond Light Source.

X-ray beam conditioning for SAXS

Karsten Joensen

JJ X-Ray Systems

The performance of a pinhole SAXS system depends critically on the choice of source, optics, pinholes, beamstops and distances. In this presentation, general guidelines for individually optimizing such performance will be presented, as well as some tricks to get the maximum flexibility out of a system once the larger design decisions have been made.

Scanning SAXS/WAXS of hierarchical fibre composites: recent experiments and future perspectives

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Advanced materials with directional mechanical properties are mostly fibre composites. They are frequently hierarchical structured, covering several length scales from the molecular or unit cell level to several hundreds of microns. The most prominent examples of outstanding hierarchical complexity are biological tissues such as bone, for instance, but also many man-made composites fall into this category. Recent developments of microbeam instrumentation at third generation synchrotron radiation sources allows to image the SAXS/WAXS signal from thin slices of such materials with a real-space resolution corresponding roughly to the beam size (i.e., down to the sub-micron regime). Apart of nanostructure mapping, in-situ mechanical testing combined with microbeam SAXS/WAXS allows to address local deformation mechanisms of composites at a specific hierarchical level.

The present contribution reviews some recent microbeam SAXS/WAXS studies of different hierarchical fibre composites. Results from the imaging of nanostructural parameters such as shape, size and orientation of fibres or particles in biological tissues are presented. Moreover, in-situ mechanical testing of single carbon fibres, and here in particular a unique combination of in-situ bending with X-ray nanobeam scanning is demonstrated. Finally, a short status report about instrumentation and experimental possibilities at the new SAXS/WAXS/Fluorescence beamline at BESSYII in Berlin will be given. A future challenge will be in particular the development of high-throughput on-line data reduction and visualization, limiting currently scanning microbeam SAXS/WAXS from being widely recognised as a real imaging technique.

Pressure-jump Studies of Liquid-crystalline Cubic Phase Transitions in Lipids

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Lyotropic liquid crystals of 1-, 2-, or 3-dimensional periodicity spontaneously assemble when biological amphiphiles are mixed with solvent under various conditions of temperature, pressure and hydration. The mesophases formed include the fluid lamellar (L/1-D), hexagonal (HI/HII/2-D) and inverse bicontinuous cubic phases (QII/3-D). Biologically, the fluid lamellar phase is ubiquitous, being the structure upon which cell membranes are based. However the inverse bicontinuous cubic phases have become increasingly accepted as not only being present in many cell membranes, but as facilitating a number of vital cell processes including endo- and exocytosis, fat digestion

and membrane budding, as these involve changes in membrane topology. These cubic phases consist of a lipid bilayer draped on mathematical surfaces known as the primitive P (space group Im3m), double-diamond D (space group Pn3m) or gyroid G (space group Ia3d) periodic minimal surfaces, subdividing 3-D space into two interpenetrating, but unconnected, water networks. The lattice parameters of these cubic phases are typically in the range 100 - 300 Å.

Previous studies of lyotropic phase transitions have concentrated on transformations between lamellar structures and between lamellar to inverse hexagonal structures, with remarkably little work being done on transitions involving cubic phases. However, a complete understanding of the physical processes governing such transitions, including the nature of any intermediates formed, and the mechanistic routes taken, is essential if we are to further our knowledge of their possible roles in fundamental cellular processes involving membranes. As a result we have recently introduced the pressure-jump technique to investigate lyotropic phase transitions by studying the rate and mechanism of the transitions in monoglyceride/water and fatty acid/phospholipid/water systems by monitoring the complete time evolution of these structural conversions. The use of pressure as a trigger mechanism has several advantages: 1) the solvent properties are not significantly altered; 2) pressure propagates rapidly meaning that equilibrium is achieved rapidly; and 3) pressure-jumps can be both in the pressurisation and depressurisation directions. A 1 kbar change in pressure typically shifts most lipid transition temperatures upwards by 20 - 30°C.

We will describe some of our recent time-resolved X-ray results obtained using beamline ID02 at the ESRF, Grenoble. Data reduction was done in part using the programs developed and implemented by Dr. P. Bösecke and colleagues at the ESRF, and in part using an IDL-based 'AXcess' X-ray analysis package developed (by A. Heron) in our laboratory in Imperial College London.

Breast Cancer Diagnosis Using Laboratory Based Small Angle X-ray Scattering, Preliminary Results.

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Previous work has indicated that breast tissue disease diagnosis could be performed using small angle x-ray scattering (SAXS) from a synchrotron radiation source. The technique would be more useful to health services if it could be made to work using a conventional x-ray source. Tumour tissue, and normal tissue from bi-lateral mastectomy procedures were examined using small angle x-ray scattering from a laboratory based source. Consistent and reliable differences in x-ray scatter distributions were observed between diseased and normal tissue samples using the laboratory based SAXSess system. Albeit from a small number of samples, a sensitivity of 100% was obtained. An encouraging result for implementing SAXS as a laboratory based diagnosis technique.

Nucleation and growth of iron oxyhydroxide nanoparticles in contaminated land environments

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Poorly ordered iron oxyhydroxide minerals (e.g. goethite, FeOOH) are a key constituent of soils and oxidised sediments. These phases control the speciation and transport of many trace components, including pollutants (e.g. arsenic), in many natural systems, due to their abundance, high surface area and reactivity. The kinetics and mechanisms of iron oxyhydroxide precipitation and crystallisation is poorly understood primarily due to their rapid formation rates (seconds – minutes), hydrated nature and nanoparticulate size which makes characterisation using traditional high vacuum ex situ techniques (e.g. TEM) difficult. In this study, we have used in situ time-resolved Small Angle X-ray Scattering (SAXS) in conjunction with a stopped-flow cell to characterise the kinetics and mechanisms of poorly-ordered iron oxyhydroxide from solution.

The initial nucleation and growth of poorly-ordered iron oxyhydroxide was studied using SAXS in conjunction with a rapid mixing stopped-flow cell on station 6.2 of the SRS (Daresbury Laboratory). With this system we are able collect scattering patterns from dilute suspension in seconds, and therefore characterise the formation process in detail. Analysis of the SAXS data indicates that the initial precipitate consists of approximately spherical or disc shaped particles <5nm in size. The particle radii increase to 20Å during the precipitation process with growth occurring predominantly in one direction by either direct precipitation or oriented aggregation. The precipitation rate increases with increasing pH, but decreases when phosphate is present due to the adsorption of the phosphate onto the growing particles.

The development of an online data analysis toolkit for x-ray scanning micro-diffraction experiments

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The Scanning X-ray micro-diffraction method has been applied to many scientific problems mainly in the field of soft condensed matter research [1]. High spatial resolution can be obtained by scanning different positions on the specimen with a micro beam which can be as small as 0.5 microns and less. The measurment positions are usually but not necessarily arranged in a grid. The data taken at each sampling point consists of two-dimensional diffraction patterns (primary images) typically recorded with a CCD-Detector (MarCCD, 2048x2048 pixels, 16 bit per sample, ~8Mb per pattern). Parameters of interest like peak positions, interated intensities, and d-spacings have to be extracted from the primary images. These values can be assembled to secondary images representing a mapping of the selected parameter on the sample. On the Quest for high resolution mappings typically huge datasets have to be recorded. About 20 Giga-Byte (2500x8Mb) of raw data have to be processed in order to obtain a 50x50 pixel secondary image. It turned out that software becomes more and more a limiting factor. Many experiments could benefit from data processing results obtained in parallel to the data acquisition. This information should be used to give feedback to the measurement control. Exposure times could be optimized. Sample regions showing interesting features can be explored in more detail and regions with low information content could be skipped.Kinetics studies could be enhanced and it would be possible to correct for sample drifts which cannot be avoided for some dynamic experiments involving mechanical deformation for instance. Many more examples could be listed here. In order to address these problems the data processing software has to exhibit "online" capability. We are currently developing a software dedicated to the kind of "online data analysis" problems sketched above. As we are dealing with a large variety of different samples and instrumental setups the software has to be very versatile and user friendly at the same time. Therefore we chose a modular aproach implementing an online data analysis kit (ODAK). In this contribution various online data analysis aspects will be discussed and the current development status of ODAKwill be presented.

[1] New avenues in X-ray microbeam experiments Riekel C. Rep. Prog. Phys. 63, 233-262 (2000)

Present Capabilities and Future Plans for Fibre Diffraction and Small-Angle Solution Scattering at the BioCAT Facility at the Advanced Photon Source

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The BioCAT undulator beamline 18ID at the Advanced Photon Source, Argonne IL., USA, was designed from the outset to be very high performance instrument for small-angle fiber diffraction and scattering. It has been very successfully used for small-angle fiber diffraction since 1998. Over the last three years we have been developing our small angle solution scattering program which is now the fastest growing user group. Another new direction is wide-angle fiber crystallography and micro-fibre diffraction. An overview of our present capabilities will be presented with selected applications. I will also present an overview of optics and detector upgrades planned for the coming year.

Non-Crystalline Diffraction at EMBL-Hamburg - Present State and Future Plans

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The X33 beamline at the EMBL-Outstation in Hamburg is a dedicated beamline for biological small angle scattering. It serves since more than 25 years a wide variety of users from structural biology for standard SAXS/WAXS experiments. Recent developments in computing of low resolution models from solution scattering allow a more detailed interpretation based on these SAXS/WAXS data. In order to fulfil the needs of the continuously growing user community the X33 beamline is upgraded during the period 2004 and 2005, implementing advanced components to increase the instrument sensitivity and resolution. In further steps the experimental setup will changed to allow a more automated operation. For this aim the existing data analysis software will be integrated and combined with the data acquisition system.

This upgrade of the X33 beamline goes in line with the plans for the new BIOSAXS beamline at the third generation synchrotron PETRA III project on the Hamburg

DESY site. The proposed SAXS/WAXS instrument will gain on the excellent beam parameters of the upgraded storage ring PETRA III and state-of-the-art X-ray optics such as multilayer monochromators, Kirk-Patrick-Baez optics will permit experiments in smaller volumes on shorter timescales. The BIOSAXS project comprises dedicated setups for non-crystalline diffraction of biological macromolecules and is part of the integrated life science center proposed by the EMBL Hamburg Outstation.

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Poster Abstracts

X-ray and Neutron scattering on soft objects in a thermotropic liquid crystal

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In a first attempt, Tanaka [1] has studied the formation of inverted micelles of didodecyldimethylammonium bromide (DDAB) plus water in the liquid crystal 5-cyanobiphenyl (5CB) as solvent. This author has claimed the existence of a new mesophase (a "transparent nematic" phase [1]) located between the isotropic and the nematic phases, which consists of random micelles embedded in a small-scale nematic matrix. This result has been controverted by Bellini [2] who has shown by a light scattering method that there was no transparent nematic phase in this system. Nevertheless, an interesting behaviour in the isotropic micellar phase is observed. With decreasing temperature, this phase is destabilized in the neighbourhood of the isotropic/nematic transition by paranematic fluctuations which give rise to an intermicellar attractive interaction.

For a better understanding, we have characterized the lyotropic organisation by X-rays and neutron scattering far above the nematic-isotropic transition of the liquid crystal. From quantitative analyses of experimental datas we obtain the shape and size of inverted micelles.

Furthermore, there are Van der Waals interactions between micelles in this system which prevent the formation of original mesophases (lamellar phase for example). We plan to add new surfactants which could reduce the VdW interactions in order to obtain original mesophases in 5CB.

References:

[1] J.Yamamoto and H.Tanaka, Nature (London) 409, 321 (2001).

[2] T.Bellini, M. Caggioni, N.A. Clark, F.Mantegazza, A.Maritan, and A. Pelizzola, Phys. Rev. Lett. 91, 8 (2003).

Phasing of Equatorial X-ray diffraction Pattern from Drosophila Indirect Flight Muscle

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The equatorial portion of a fiber diffraction pattern gives information on the projection of the electron density onto a plane perpendicular to the long fiber axis. In the muscle community, changes in the intensity of the 1,1 equatorial reflection relative to the 1,0 reflection (I11/I10) in vertebrate muscle or the I2,0 /I10 in insect muscle have long been used as a qualitative measure of changes of the relative amount of crossbridge mass associated with the thin filament relative to the thick when muscles change state. If the phases of the higher order equatorial reflections can be estimated, electron density maps may be calculated allowing a more sophisticated level of analysis. The indirect flight muscle of Drosophila is a highly ordered system giving up to 14 equatorial reflections (5,1) or about 90 nm resolution. This is too low a resolution to give useful filament dimensions but may give qualitative indications about changes in dimensions. Since electron density maps are based on all the available be intensity data can yield more realistic estimates of mass shifts than I20/I10. Here we approached the phase problem using a combination of methods. The first method was to approximate the equatorial projection of the electron density as cylindrical regions of electron density with electron densities in various regions based on up to date literature values for lattice spacing along with the geometry of the unit cell. At this low resolution, centro-symmetry can be assumed and relatively crude models can be justified. By Fourier transforming these density distributions, the predicted intensities can be calculated and fit to experimental data using a Differential Evolution (DE) algorithm to minimize chi-squared. The simulation gives predicted phases for the reflections that were compared to those obtained from Fourier transformation of electron micrographs. These two phase sets were found to differ only for the (2,1), (2,2), and (5,0) reflections (which are all weak) To resolve the phase differences, electron density maps were calculated for each set of phases (EM and simulation) for data taken from skinned fibers at varying degrees of lattice spacing. The simulation phase set gave more realis-

tic changes in electron maps with lattice shrinkage than the EM phase set. Electron density maps showed a hollow center in the thick filaments using the simulation intensities and phasing. The thick filament appeared to be compressed at high degrees of lattice compression probably due to the compressibility of the hollow core. The radial extent of the myosin heads also was reduced with increasing lattice shrinkage. The simulation predicts that the crossbridges are not near the actin filaments during the relaxed state and a uniform distribution of myosin heads around the backbone. Temperature factor type disorder in the thick and thin filament was very low. Supported by the U.S. National Institutes of Health.

X-Ray Diffraction experiments to study the structural ordering of hybrid organic-inorganic sol-gel materials

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Powder X-ray diffraction experiments have been performed to analyse the structural order of urea-based alkylene bridged silsesquioxanes with various carbon chain lengths. The results show that the organisation of the organic fragments in these hybrid silica is greatly influenced by the increasing length of the alkylene chain, the H-bonding of the urea groups and depends also on the reaction conditions.

Recent results from in/ex-situ x-ray microdiffraction studies of microdeformation induced by indentation applied to polymer fibres

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The results presented in this poster demonstrate, for the first time, the feasibility of combined studies of microindentation and scanning X-ray microdiffraction (WAXS) techniques in-situ (real-time)[1]. For this purpose, a dedicated microindentation device was developed on the microfocus beamline of the ESRF (ID13). First experiments were focused on polymer fibres even though the method can also be applied to other materials such as biomaterials or metals. In-situ experiments showed that at least a part of the crystalline domains within the volume probed in the X-ray beam tend to orient in the stress field induced by the indenter during deformation (i.e. while the load is applied)[1]. Furthermore, the original crystalline orientation is almost fully recovered upon releasing the stress on the diamond tip, and should therefore be associated with elastic relaxation. Ex-situ experiments, on the other hand, showed that the orientation is partly retained in the immediate vicinity of the tip where the stress is highest, giving rise to strong textures[2,3]. Another important consequence of indentation was found in the form of phase transformations occurring in two high-performance fibres[1,2,3]. Particular emphasis is put on recent results obtained using Vectra liquid-crystalline fibres [4].

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[3] M.C. Garcia, A. Gourrier, C. Riekel, J. Macro. Sci., B 43, 267, 2004
[4] A. Gourrier, M.C. Garcia, C. Riekel, Macromolecules, 38, 3838, 2005

The polymer network of wood cell walls: an examination by small-angle X-ray scattering and X-ray diffraction

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The three structurally relevant groups of polymers in wood cell walls are cellulose, lignin and hemicelluloses. Our aim is to gain more insight into the function of hemicellulose and lignin, the matrix polymers in which the cellulose fibrils are embedded. Slices of spruce wood (Picea abies [L.] Karst.) were subjected to chemical treatments, which degrade and remove lignin and hemicelluloses to varying degrees and, as a consequence, have a specific effect on the swelling behaviour and aggregation of the cellulose in the cell wall. The used chemical agents were sodium chlorite (NaClO2) for delignification, sodium hydroxide at different concentrations for removal of hemicelluloses and an acidic hydrogen peroxide solution, which removes most of the cell wall matrix. The corresponding structural changes of the cell wall were observed by small-angle scattering (SAXS) and wide-angle diffraction (WAXS), in the wet (never dried) state and after drying. While mere delignification had only little effect on the swelling and drying behaviour of the cellulose fibrils, the structural changes upon increasing degradation of the hemicelluloses were severe.

SAFs: self-assembled protein fibres of de novo design

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Self-assembling systems are found extensively in biology, An improved understanding of these would fuel efforts to engineer novel, bioinspired materials through de novo design. Coiled-coil motifs provide simple systems for studying molecular self-assembly. We have designed two 28-residue peptides to assemble into extended coiled-coil fibres. Complementary peptide-peptide interactions were used to direct the folding of a "sticky ended" heterodimer as a building block for the assembly of long fibres. Assembly was monitored in solution using circular dichroism spectroscopy. Furthermore, fibre formation was confirmed by electron microscopy, which revealed linear non-branched structures, tens of μm long and ~50 nm thick. This thickness is ~20 greater than expected for the two-stranded coiled-coil target. One explanation is that the two-stranded structures are nascent protofibrils that bundle to form the matured, thick fibers. X-ray fibre diffraction of partially aligned samples gave patterns indicative of coiled-coil structure, and also suggested that the protofibrils packed to form a highly ordered, hexagonal lattice. The use of fluorescently labelled peptides showed that the fibres were polar, as the labelled molecules incorporated specifically to one end of the fibres. Redesign of the peptides resulted in an increase in order, thickness and thermal stability of the fibres. Furthermore, the design of non-linear peptides based on the original peptide has provided insight into how fibre morphology can be controlled. These findings show that the self-assembled fibres are rich in structure and information, and that they present possibilities for the bottom-up assembly of new nanostructured functional biomaterials.

Time Resolved X-ray Diffraction Studies of Active Bony Fish Muscle: Analysis using the New CCP13 Program FibreFix

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The well known swinging crossbridge model of muscle contraction was first put forward in 1969 by Hugh Huxley and since then a large body of work has built up in support of this theory. However, there are certain aspects of the theory which require more clarification, for example: what are the occupancies and kinetics of the different states of the crossbridges during this cycle To this end, this project is employing time-resolved X-ray diffraction to intact, active bony fish muscle. X-ray diffraction has the advantage over other methods of studying muscles in that it can be used on live contracting muscle specimens whilst still providing data with high spatial resolution [Squire et al., 2003]. Bony fish muscle is being used in conjunction with X-ray diffraction because it is very well ordered compared to other vertebrate muscles such as frog, showing long range order and a simple 3-D cross-bridge lattice.

Previous work carried out on bony fish muscle has looked at the changes which occur in the X-ray pattern during contraction in conjunction with the tension timecourse produced by the muscle. Up until now the effects of any sarcomere length change which might occur during the contraction of this muscle have been neglected because the change was thought to be small [Harford & Squire, 1992]. However, sarcomere length changes can have an effect on both the timecourse of tension development and the timecourse of intensity changes of the X-ray reflections from the muscle. Therefore, it is important to be able to measure and control the sarcomere length change which occurs during contraction of bony fish muscle to quantify these effects.

With this in mind, a sarcomere length control system has been developed based on monitoring muscle sarcomere length using laser diffraction. The system can measure the sarcomere length change accompanying contraction and can also reduce this change by about half, whilst X-ray data are being gathered. This control has had an effect on the tension timecourse. X-ray diffraction data from muscles under partial sarcomere length control and in improved physiological conditions have been obtained and are now being analysed using the new integrated CCP13 program FibreFix. New analysis tools have been added to this program, making the processing of time resolved data much easier and more efficient [Rajkumar et al., 2005]. This poster demonstrates some of the new functionality of FibreFix applied to the latest X-ray data from bony fish muscle under partial sarcomere length control.

Harford, J.J. & Squire, J.M. (1992) "Evidence for structurally different attached states of myosin crossbridges on actin during contraction of fish muscle" Biophys. J. 63: 387-396

Squire, J.M., Knupp, C., AL-Khayat, H.A. & Harford, J.J. (2003) "Millisecond time-resolved low-angle X-ray diffraction: a powerful, high-sensitivity technique for modelling real-time movements in biological macromolecular assemblies." Fibre Diffraction Review 11: 28-35.

Rajkumar, G., AL-Khayat, H., Eakins, F., He, A., Knupp, C. & Squire, J.M. (2005) "FibreFix - A New Integrated CCP13 Software Package" Fibre Diffraction Review (in press).

Nanoscale assemblies of aligned polyfluorene films

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We report on the design of anisotropic structures of liquid crystalline pi-conjugated polymer, PF2/6 polyfluorene, including a subtle issue of crystal orientation. At a threshold molecular weight Mn*10 kg/mol, PF2/6 has a phase transition between hexagonal and nematic phases[1] separating low (Mn Mn*) and high (MnMn*) molecular weight regimes which affects both alignment[2] and surface morphology[3]. In aligned films on rubbed polyimide, the rigid PF2/6, taking the helical axes parallel to crystallographic c axis, lies on the substrate pointing in the rubbing direction (denoted z here). The aligned film shows two differently oriented coexisting crystallite types in-plane. Type I have crystal axis a normal to the surface (x) and type II along the substrate surface (y)[4-5]. This organization leads to a large absorption and dispersion of the refractive index in the (xy0)-plane with respect to the x axis[6].

[1] Knaapila et al. Phys. Rev. E. (2005) 71 041802
[2] Knaapila et al. Macromolecules (2005) 38 2744
[3] Knaapila et al. Adv. Funct. Mater. (2005) in press
[4] Knaapila et al. J. Phys. Chem. B (2003) 107 12425
[5] Knaapila et al. J. Phys. Chem. B (2004) 108 10711
[6] Lyons & Monkman J. Appl. Phys. (2004) 96 4735

Sulphotransferase mutations and corneal matrix structure

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4. Otsuka GEN Research Institute, Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan.
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Keratan sulphate (KS) glycosaminoglycans (GAGs) substituted on proteoglycans (PGs) are key constituents of the extracellular matrix of the corneal stroma where they are believed to help govern collagen fibrillar ultrastructure. Corneas of mice lacking the KSPGs lumican and keratocan show structural matrix changes, whereas the corneal stroma in mice with a null mutation for the other corneal KSPG, mimecan, is minimally affected. To assess the role of KS GAG sulphation in the control of collagen matrix structure in cornea we generated mice with null mutations in Chst5, a gene encoding an N-acetylglucosamine-6-O-sulphotransferase (GlcNAc6ST) that is integral to the sulphation of KS chains. This gene is an ortholog of CHST6, which in humans encodes for a GlcNAc6ST and is causative for macular corneal dystrophy (MCD), a recessive condition characterised by progressive corneal clouding. Chst5+/- ES cells were generated by homologous recombination using a target vector that contained a genomic DNA fragment of Chst5 with a neo expression cassette which replaced a protein encoding exon of the gene. Chst5 null mice were generated by intercrossing Chst5+/- mice originated from the targeted ES cells. Corneas of Chst5 null mice were optically clear, and even in old age showed no evidence of a murine form of MCD. The collagen fibrillar architecture of corneas from 21 mature (5-10 month) mice was studied by synchrotron x-ray fibre diffraction, and this disclosed that average collagen fibril diameters in heterozygous (35.7nm ± 0.6nm; n11), homozygous (34.9nm ± 0.7nm; n18), and wild type (36.4nm ± 0.9nm; n12) corneas were not significantly different. Average centre-to-centre fibril spacing in corneas of homozygous mutants (42.5nm ± 3.4nm; n18), on the other hand, was lower than that in wild type corneas (47.8nm ± 3.5nm; n12) and heterozygous corneas (48.3nm ± 2.2nm; n11), and these differences were highly significant (p0.001). Local order in the fibrillar array, as indicated by the coherence distance, was lower in corneas of homozygous mutants (184nm ± 19nm; n18) than heterozygous mutants (236nm ± 13nm; n11) or wild types (247nm ± 18nm; n12). Thus, despite the fact that KS in mouse cornea has less highly sulphated epitopes than KS in the corneas of other, larger mammals the discovery of structural matrix changes in homozygous Chst5 null mice points to a role for KS sulphation in the control of collagen fibril arrangement in mouse cornea.

An X-ray Diffraction Study of Collagen Orientation and Mass Distribution in Normal and Keratoconus Corneas

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Purpose:
To increase our understanding of the relationship between corneal structure and shape, we examined the structural abnormalities associated with the disease keratoconus. Keratoconus is characterised by a thinning and steepening of the cornea.

Methods:
Three keratoconus corneal buttons of 8mm diameter and three normal human corneas were tagged with a nylon suture at the 12 o'clock position, before being preserved in 10% formalin. A videokeratographic image of surface dioptric power was recorded for each cornea (in vivo for keratoconus corneas and in vitro for the normal controls). Wide angle x-ray scattering (WAXS) patterns were obtained at 0.4mm intervals over the entire area of each sample using a computer operated translation stage on Station 14.1 at the Daresbury Synchrotron Radiation Source, UK. Each WAXS pattern was analysed to produce quantitative information regarding the total amount of collagen (aligned and isotropic) and the preferred orientation of aligned collagen at a known position in the cornea. By arranging the data onto a grid of corneal position, various maps were produced to illustrate the distribution and preferential orientation of collagen in each sample. The relationship between collagen arrangement and surface topography was examined in detail for both the normal and keratoconus corneas.

Results:
The preferred orientation of collagen and the distribution of collagen mass was altered in the keratoconus corneas, and in each case the nature of the abnormalities appeared to be related to the surface topography. In the apical region of the diseased corneas, the normal orthoganol preferred orientation of collagen fibrils was absent. Also, in contrast to the normal gradual symmetrical increase of collagen from the central cornea to the periphery, maximal thinning occured in the apical region of the keratoconus corneas and an asymmetrical distribution of collagen was seen throughout the remainder of each button.

Conclusion:
The results indicate a redistribution of collagen mass in keratoconus corneas. This study therefore supports the theory that corneal thinning in keratoconus occurs as result of lamella sliding away from the apical region. The existence of this mechanism would also help to explain the altered orientation of collagen fibrils in this region.

Collagen Interfibrillar Spacing in the Developing Chick Cornea and the Influence of Keratan Sulphate

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PURPOSE. In the week leading up to hatch, the embryonic chick cornea thins and becomes transparent (Coulombre and Coulombre, 1958; Hay and Revel, 1969; Connon et al., 2002, 2003). Proteoglycans have long been envisaged as potential modulators of corneal structure in the latter stages of development; previous chemical quantification of glycosaminoglycans from corneal isolate has indicated no change in the amount, molecular size, or degree of sulphation between developmental days 10 and 14 (Hart, 1976). After this time keratan sulphate (KS) becomes more highly sulphated, with lumican likely bearing most of these chains (Cornuet et al., 1994; Dunlevy et al., 2000). This study aims to correlate changes in the levels of sulphated KS with collagen interfibrillar spacing in the developing chick cornea.

METHODS. Low-angle synchrotron x-ray diffraction of 78 isolated corneas from chicken embryos obtained daily from developmental day 12 to day 18 (n10 to 12 at each timepoint) was used to non-invasively measure average centre-to-centre collagen fibril spacing. Next, quantification by ELISA of papain digests of the same corneas was performed using the monoclonal antibodies 5D4 and 1B4 that recognise highly and lesser sulphated epitopes on the KS chain respectively.

RESULTS. Antigenic highly sulphated KS in the developing chick cornea (given as ug/mg wet weight of tissue (+/-SD)) measured 2.7+/-1.5 (day 12), 3.1+/-0.7 (day 13), 2.0+/-0.8 (day 14), 2.6+/-1.5 (day 15), 11.6+/-4.4 (day 16), 16.1+/-8.4 (day 17), and 24.1+/-7.9 (day 18) as quantified by 5D4. Lesser sulphated KS measured using 1B4 undergoes little change in the earlier stages of development; between days 12 and 15 levels rise from 0.29+/-0.35 to 0.52+/-0.34. After this point, levels increase more rapidly; doubling from 0.73+/-0.42 (day 16) to 1.50+/-1.04 (day 18).

Over the period studied the average collagen fibril spacing in these same corneas dropped from 65nm to 53nm, and mainly this occurred after day 15. For the data set of 78 individual corneas, collagen interfibrillar spacing and antigenic 5D4 KS levels showed a significant inverse correlation (p0.001; R20.501), accompanied by a non-significant rise in lesser sulphated KS as measured by 1B4 (p0.005, R20.3897).

CONCLUSIONS. As the secondary chick cornea develops and becomes transparent the compaction of stromal collagen fibrils is accompanied by an increase in tissue levels of sulphated KS. The remodelling of fibrillar arrangement may be related to a switch to highly sulphated KS production, however KS is unlikely to be the sole driver of this transition, which we feel suggests a possible role in "fine tuning" the fibrillar array.

Small Angle X-ray Scattering Studies of Collagen Degradation

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Recent studies have investigated the sensitivity of Small Angle X-ray Scattering (SAXS) data to detect changes in the extracellular matrix (ECM) structure in breast cancer.

Systematic differences in the axial d-spacings and intensities of X-ray scattering from the fibrillar collagens in malignant, benign and normal tissues of the human breast have been observed. These differences are hypothesised to arise from the remodelling of the ECM that is necessary for tumour growth, invasion and metastasis. These events are associated with abnormal expression of the collagenases responsible for ECM degradation. We observed the in vitro enzymatic degradation of rat tail collagen, porcine subcutaneous tissue and human breast tissue using SAXS in an attempt to further understand the ultrastructural changes that take place during breast cancer progression. The results suggest that the intensity changes previously observed in malignant tissues are the result of degradation processes but that the axial spacing changes arise from abnormal collagen formation.

2D Model Fitting to SAXS Patterns from Soft Tissue

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Here, methods are presented to achieve 2D chi square fitting to specific features which in this case are ordered collagen peaks along with a representation of the background scatter. Requirements for much of the analysis required from soft tissues small angle x-ray scattering (SAXS) patterns, producing parameters is used to describe and simplify the data. Radially averaged 1D data is obtained. Curve fitting is then employed on resulting 1D data to extract relevant information as part of the analysis procedure. Here a simple model was used and reliably fitted to a variety of 2D diffraction patterns for which the log was first taken to reduce the data range and improve fitting accuracy. The model used to fit to log data consisted of an exponential background along with a specified number of Gaussians. When used with the 2D case the model becomes rotated about a specified centre. To achieve a reliable fit for the model, estimates from a 1D averaged sector were fitted.

The 1D curve fit estimates were supplied to the 2D fitting and with the aid of sensible data weighting and basic wavelet filtering, successful and reliable fitting of a specified 2D model is shown to be achievable. In this way multiple patterns could be fitted without the need for time consuming data minimizing and laborious curve fitting. As described the model is very simple and allows area data to be parameterized to a minimal form to extract ordered collagen information such as position. The simple model can easily be extended to achieve a more comprehensive and complex pattern fitting achieving orientation distribution functions.

An X-ray Diffraction Study on Mouse Cardiac Cross-Bridge Function in vivo : Effects of Adrenergic Beta-stimulation

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X-ray diffraction from cardiac muscle in the left ventricular free wall of a mouse heart was recorded in vivo. After the R-wave in electrocardiogram, the ratio of the intensities of the equatorial (1,0) and (1,1) reflections decreased for about 50 msec from a diastolic value of 2.1 to a minimum of 0.8, and then recovered. The spacing of the (1,0) lattice increased for about 90 msec from the diastolic value of 37.2 nm to the maximum of 39.1 nm, and then decreased. Stimulation of beta-adrenergic receptor by dobutamine accelerated both the decrease in the intensity ratio, which reached a smaller minimum ratio, and the increase in the lattice spacing, but the intensity ratio and spacing at the end-diastole were unchanged. The recovery of the lattice spacing during relaxation was also accelerated. These results support the current view that beta-stimulation accelerates both activation and relaxation in cardiac muscle. This is the first in vivo investigation at a molecular level on how β-stimulation affects the contractility of cardiac muscle. This x-ray diffraction technique will be an excellent method to examine left ventricular contractility in live transgenic mice.

Small Angle X-ray Scattering of a lobster aorta

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Cardiff University, School of Optometry and Vision Sciences, Biophysics Group

The aorta of primitive vertebrates and invertebrates have been shown to contain fibrillin-like microfibrils, implying that these biopolymers have had an elastic role for at least 550 million years. In mammalian tissues, fibrillin molecules assemble to form microfibrils. It remains unclear whether the important mechanical and elastic roles of fibrillin in tissues across the animal phyla occur at a molec-

ular or a suprafibrillar level. Small-Angle X-ray diffraction techniques were used to determine and compare the structure-function relationships in tissues with a phylogenetic distance. A segment of the abdominal artery of a lobster was mounted on a mechanical rig to allow tissue extension and the biomechanical testing was combined with Small-Angle-X-Ray-Scattering (SAXS) at ID02 at the European Synchrotron Radiation Facility. The fundamental axial periodicity in the unstretched lobster aorta was found to be 45nm against 56nm in the mammalian tissues. The axial periodicity increased by up to 150% in the lobster aorta against 87% in the mammalian zonular filaments, indicating a much higher elastic extension limit. The presence of meridional series and an equatorial scatter corresponding to a lateral interference function of 37nm in the unstretched lobster aorta indicated the presence of an axial periodic structure. Wess et al. (1998 and 1997) have also reported the existence of a periodic structure in the mammalian zonules. The lobster tissue extension led to a gradual shift of the lateral spacing towards higher reciprocal distance values, indicating an increased lateral packing density. When the lobster aorta tissue was tensioned by up to 70%, the axial coherence decreased, as the fibrillin-like microfibrils most probably reorganised. At a tissue stretching of 100%, the predominance of the third order in the meridional series showed an ordered microfibrillar arrangement, possibly with a one-third stagger. At tissue stretching of 130%, however, this microfibrillar arrangement was much less obvious. In the mammalian zonules, the observed third meridional peak has been found to disappear as the tissue is stretched (Haston et al., 2003). Therefore there are both commonalities and differences in the biomechanical responses of mammalian and invertebrate microfibrils-containing tissues.

Development of FibreFix A new Integrated CCP13 software package

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A new Integrated CCP13 program FibreFix has been developed which incorporates the functionality of the CCP13 analysis programs XCONV, XFIX, FTOREC and LSQINT and also many important functionalities of the well-known BSL program.

FibreFix is now a complete, user-friendly, program for X-ray fibre diffraction data stripping. It incorporates a new version of LSQINT, which has been completely redeveloped with a graphical user interface (GUI), and also provides direct access to the helical diffraction simulation programs HELIX and MusLABEL

For further details of the FibreFix program see:

Rajkumar, G., AL-Khayat, H.A., Eakins, F., He, A., Knupp, C. & Squire, J.M. (2005) FibreFix - A New Integrated CCP13 Software Package. Fibre Diffraction Review 13, 11-18.

Also see tutorials on the CCP13 website: www.ccp13.ac.uk

An X-ray Diffraction Study of Collagen Orientation and Mass Distribution in Normal and Keratoconus Corneas.

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Purpose:
To increase our understanding of the relationship between corneal structure and stability we examined the structural abnormalities associated with the disease keratoconus. Keratoconus is characterised by a thinning and steepening of the cornea.

Methods:
Three keratoconus corneal buttons of 8mm diameter and three normal human corneas were tagged with a nylon suture at the 12 o%27clock position, before being preserved in 10% formalin. A videokeratographic image of surface dioptric power was recorded for each cornea (in vivo for keratoconus corneas and in vitro for the normal controls). Wide angle x-ray scattering (WAXS) patterns were obtained at 0.4mm intervals over the entire area of each sample using a computer operated translation stage on Station 14.1 at the Daresbury Synchrotron Radiation Source, UK. Each WAXS pattern was analysed to produce quantitative information regarding the total amount of collagen (aligned and isotropic) and the preferred orientation of aligned collagen at a known position in the cornea. By arranging the data onto a grid of corneal position, various maps were produced to illustrate the distribution and preferential orientation of collagen. The relationship between collagen arrangement and surface topography was examined in detail for both the normal and keratoconus corneas.

Results:
The preferred orientation of collagen and the distribution of collagen mass was altered in keratoconus corneas; the abnormalities appeared to be related to the specific shape of each cornea. In the apical region of the keratoconus corneas, the normal orthoganol preferred orientation of collagen fibrils was absent. Also, in contrast to the normal gradual symmetrical increase of collagen from the central cornea to the periphery, maximal thinning occurred in the apical region of the keratoconus corneas and an asymetrical distribution of collagen was seen throughout the remainder of each button.

Conclusion:
The results indicate a redistribution of collagen mass in keratoconus corneas. This study therefore supports the theory that corneal thinning in keratoconus occurs as result of lamella sliding away from the apical region. The existence of this mechanism would also help to explain the altered orientation of collagen fibrils in this region.

Application of the new Integrated CCP13 Software Package FibreFix on an amyloid X-ray diffraction pattern

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The new, single, user-friendly CCP13 software package known as FibreFix (Rajkumar et al., 2005, Fibre Diffraction Review 13, 11-18) has been applied for the first time to an X-ray fibre diffraction pattern from amyloid fibrils formed from fragment A(11-25). This construct gives a distinctive X-ray diffraction pattern and has been analysed previously (Sikorski et al. 2003, Structure 11, 915-926) using different software for stripping the X-ray diffraction patterns. The pattern is sampled, but highly disoriented. Here we describe in detail the steps involved in using FibreFix in analysing such an amyloid X-ray pattern. This involved, centering the pattern, determining its rotation, defining the specimen tilt, fitting the background, subtracting the background, obtaining peak intensities values and defining the unit cell parameters. The intensity values can in future be used in further modelling analysis to study the arrangement of the peptide within the amyloid fibre specimen for comparison with the previously published model of Sikorski et al. (2003, as above).

Original amyloid X-ray data from Dr. Louise Serpell with permission.

Mouse Corneal Development

Jack Sheppard, Keith Meek, Marcela Votruba

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Murine corneal development in terms of collagen development and collagen orientation has not yet been studied. Transparency is dependent on collagen orientation. Developing murine corneas were studied at time points post natal days 7-11 to see how collagen orientation and collagen mass develop. This worl is in conjuction with looking at how the development of the cornea occurs during normal development and will later be compared to the developing corneas of keratoconic mice.

Electrospinning triblock Copolymer Ultrafine Fibers

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Electrospinning is a straightforward method to produce ultra-thin fibers (tens of manometers to several microns) from polymer solutions. It could take advantage of the high surface to volume ratio to make very efficient sensors and nanotechnology devices.

In this study, he linear triblock copolymers (Kraton G1650) was solved in tetrahydrofuran (THF), and the ultrafine fiber are produce by the electrospinning the polymer solution. The morphologies and the structure properties of the polymer solutions and the electrospun fibers were investigate and compared by small-angle X-ray scattering (SAXS).

Metallocene made Isotactic Polypropylene: Flow Induced Crystallisation and Structural Transformation during Stretching

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Metallocene catalysts enable isotactic polypropylene (i-PP) to be prepared with well-defined levels of defects in terms of stereoregularity. We have used in-situ synchrotron-based small angle X-ray scattering (SAXS) experiments to probe the behaviour of these novel polymers under shear flow and during the subsequent crystallisation and during deformation in the solid state.

The SAXS data shows the development of oriented structures perpendicular to the flow direction (kebabs) and we explore the effect of different amounts of defects of stereoregularity on the crystallisation of i-PP under the influence of a shear flow field is explored. Wide angle X-ray scattering (WAXS) patterns of samples crystallised after cessation of the shear flow indicate a significant level of preferred orientation of the crystals. Moreoever, the WAXS data reveal that these structures contain disordered modifications intermediate between the a and g forms.

It has been recently shown that metallocene made i-PP samples containing high concentration of defects exhibit high levels of elasticity. The elastic recovery is associated with a reversible polymorphic transition between the mesomorphic and the a form. In-situ synchrotron-based WAXS diffraction data obtained during stretching and relaxation cycles of an i-PP sample with a high concentration of defects have shown that the g form present in the unstretched film transforms into the mesomorphic form at high deformation. The mesomorphic form transforms into the a form by releasing the tension. This transition is accompanied by an elastic recovery. For samples containing a lower level of stereoregular defects, which do not show elastic properties, no polymorphic transition is observed after releasing the tension and no elastic recovery is observed.

The use of a low molar mass self assembled template to direct the crystallisation of Poly(e-caprolactone)

Supatra Wangsoub, Geoffrey R Mitchell, Robert H Olley

University of Reading

We show that small quantities of dibenzylidene sorbitol dispersed in poly(e-caprolactone) coupled with shear flow provide a self-assembling nanoscale framework to yield high levels of crystal orientation. Under shear flow, the additive forms highly extended nano-particles which lie parallel to the flow field and on cooling, polymer crystallisation is directed by these particles. We use in-situ time-resolving SAXS and WAXS techniques to explore how this behaviour can be modified by the composition, the shear rate and strain. In-situ SAXS measurements show that during the flow an anisotropic structure develops with highly extended objects of length ~700%C5 in the flow direction and ~40-60 %C5 in the transverse direction. On cooling the SAXS patterns obtained at room temperature show the presence of a highly anisotropic lamellar structure with a long spacing of ~180%C5. The corresponding WAXS patterns show significant anisotropy in the 110 and 200 crystalline peaks. Although 1% of DBS is sufficient to produce these templating effects, increasing the level of DBS leads to increased anisotropy in the PCL crystals until the effects plateau at ~ 3%. Increasing the shear rate for the flow imposed in the melt state leads to high levels of anisotropy of both the DBS fibrils in the melt and the templated PCL at room temperature. In addition, there is an increase in the level of anisotropy with increasing shear strain with some evidence for a plateau at the highest shear strain.

In conclusion, the combination of DBS and shear flow leads to an overall morphology with a high level of PCL crystal orientation. Without the additive the PCL exhibits an isotropic microstructure. This templating represent a novel and powerful approach to effective microstructure control.

Layer Specific Collagen Orientation in Human Arteries During Tensile Testing

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The collagen diffraction patterns of human aortas under uniaxial tensile test conditions have been investigated by synchrotron small angle X-ray diffraction. Using a recently designed tensile testing device the orientation and d-spacing of the collagen fibers in the major arterial layers have been measured in situ under physiological conditions together with the macroscopic force and sample stretching. The results show a direct relation between the orientation/extension of the collagen fibers on the nanoscopic level and the macroscopic stress and strain. This is attributed first to a straightening, second to a reorientation of the collagen fibers, and last to an up-take of the increasing loads by the collagen fibers.

[1] F. Schmid et al., In Situ Tensile Testing of Human Aortas by Time-Resolved Small Angle X-ray Scattering, J.Synchr.Rad., 2005, in press.
[2] F. Schmid et al., In Situ Tensile Testing of Human Arteries, in: Elettra Science Update, April 2005, http://www.elettra.trieste.it/science/update/docs/SAXS_050323_Arteries.pdf